

### REMARKS

Claims 1-13 were previously pending in this application. By this amendment, claims 1, 2, 12, and 13 have been amended. As a result claims 1-13 are pending for examination with claims 1 and 13 being independent claims. No new matter has been added.

#### Rejection under 35 U.S.C. §112

Applicant traverses the rejection of claims 1-13 under 35 U.S.C. §112, second paragraph, as the present claims, as amended, are not indefinite. Claims 1, 2, 12, and 13 have been amended to further clarify Applicants' claimed invention and to overcome this rejection.

Specifically, to address the objection to claims 1, 6, 10 and 13, the initial and final amounts for the steps of "increasing" or "decreasing" calcium are provided. Support for these amendments is found at pg. 6, ln. 16; pg. 6, ln. 23; and pg. 6, lns. 34-35.

The Examiner's question concerning the steps in the process is addressed in the specification at pg. 5, lns. 11-20, and in the claims themselves. As stated there, the cells are suspended repeatedly (or successively) in a first or second solution. The composition of each solution remains the same, except that the amount of  $\text{CaCl}_2$  is increased or decreased between one repeat and the next.

Concerning the differences between claim 1(b) and claim 6, claim 1(b) is directed to decreasing the amount of  $\text{CaCl}_2$ , and claim 6 is directed to decreasing the concentration of the  $\text{CaCl}_2$ .

Concerning the differences between claim 1(d) and claim 10, claim 1(d) is directed to increasing the amount of  $\text{CaCl}_2$ , and claim 10 is directed to increasing the concentration of the  $\text{CaCl}_2$ .

The use of Earle's is intentional. Eagle's medium is not used.

Concerning claims 12 and 13, the specified concentrations of the components are considered to be presented in standard terms of weight and measure. Accordingly, one skilled in the art would be able to make any adjustments to reach a desired volume.

Accordingly, withdrawal of the rejection of claims 1-13 under 35 U.S.C. §112, second paragraph, is respectfully requested.

Rejections Under 35 U.S.C. §103

Applicants traverse the rejection of claims 1-13 under 35 U.S.C. §103(a) as being obvious in view of Kruppenbacher, et al., Kang, et al., and the ATCC excerpt. As noted above, Applicants do not use Eagle's media, and therefore the ATCC excerpt does not apply.

Kruppenbacher, et al. does not teach or suggest the whole of Applicants' claimed invention, nor does Kruppenbacher et al. teach or suggest the need to combine its teachings with those of Kang, et al. As amended, Applicants' claims are directed to isolating adult cardiac cells, while Kang, et al. are directed to neonatal cardiac cells. The methodologies are distinct, as the references themselves make clear.

Applicants' claimed invention is directed to a method of isolating adult cardiac cells. The method includes the steps of successively exposing cardiac tissue to a first solution in which the amounts of  $\text{CaCl}_2$  are decreased from about 1-2  $\mu\text{M}$ . The first solution is made with NaCl, HEPES,  $\text{MgCl}_2$ , KCl, and sugar to a pH of approximately 7.4. The tissue is dissociated with an enzyme solution, and the disassociated tissue is resuspended into a second solution with amounts of  $\text{CaCl}_2$  that are increased from about 1-2  $\mu\text{M}$ . The second solution comprising modified Earle's, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, and an antibiotic, to a pH of approximately 7.4. The cells can be incubated in a mixture of carbon dioxide and air, and resuspended.

As the Examiner notes, Kruppenbacher does not teach or suggest use of HEPES, nor does the reference teach the use of modified Earle's. Likewise, the concentrations of Applicants' particular ingredients are not disclosed. As Kruppenbacher, et al. makes clear, he found that his specific ingredients, concentrations, and conditions, were all critical to success (pg. 133, col. 1). Moreover, Kruppenbacher, et al. teaches that interchanging media, as the Examiner suggests, is not advantageous. pg. 134, col. 1. Applicants' claimed invention is, therefore, not inherently obvious.

As discussed above, Kang et al. is directed to neonatal cardiac myocytes, not to adult cardiac cells. Thus, there is no motivation to combine the two references. Furthermore, the use of HEPES in this reference is in a solution used to make contractility measurements, not to isolate or culture the cells. (pg. 9886, col. 2).

Accordingly, withdrawal of this rejection is respectfully requested.

**CONCLUSION**

In view of the foregoing amendments and remarks, claims 1-13 of this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the Applicant's attorney at the telephone number listed below.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 50/2762.

Respectfully submitted,  
*Attorney for Applicants*

By: 

Ann Lamport Hammitte, Reg. No. 34,858  
LOWRIE, LANDO & ANASTASI, LLP  
One Main Street  
Cambridge, Massachusetts 02142  
United States of America  
Telephone: 617-395-7000  
Facsimile: 617-395-7070

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